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Vol. 70 No. 23 Issue No. 4183 465-486

June 7th 1958

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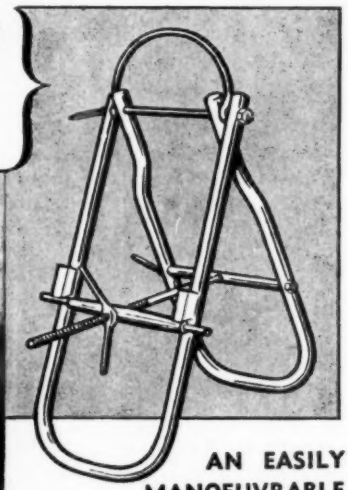
BAGSHAWE'S Cattle Hoist

(Pat. Pending)



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No. 23 JUNE 7th, 1958 Vol. 70

Registered for Transmission as a Newspaper *Price 2s. post free*

Scientific Letters to the Editor

RECENT correspondence published in the columns of THE VETERINARY RECORD has referred to the practice of making brief reports on research findings in the form of "Letters to the Editor." Such reports may be of various kinds: they may be intended to stand as the only published record of some piece of work that is of such a nature as to be adequately reported within the compass of a thousand words; they may represent an account of some relatively trivial or minor observation confirming or modifying previous scientific publications, or they may represent a brief summary of completed work that is to be published in full at a later date.

This last is perhaps the most important function for these brief contributions to fulfil. It will be agreed that prompt publication of research findings is the very life blood of active research and that one of the first duties of a research worker is to see that his colleagues are made aware of what he has added to existing knowledge. To make original research observations and not to publicise them is a waste of research effort.

For this purpose it is thought that the form of a "Letter to the Editor" is particularly well suited, as the personal approach encourages a direct form of treatment, such as leads both to clarity and brevity and suggests the alliance and colleagueship that is conducive to good co-operation. It is a method that has been well tried, going back as it does through the 88 years of *Nature*, which first appeared in 1869 with scientific correspondence in the very first number, since when the feature has been of ever-increasing importance.

Many other scientific periodicals have recently followed this lead and it is the opinion of the Editorial Committee that THE VETERINARY RECORD should recognise the advantages and adopt the procedure. This has been thoroughly well discussed privately among a number of research people, by the Editorial Committee, and at meetings of the English branches of the Association of Veterinary Teachers and Research Workers and has met with unanimous approval. The Editor, therefore, now invites veterinary research workers to contribute brief reports of their observations, similar to those which appear in *Nature*, and differing only in that they will be of the veterinary specialisation. Writers should address the Editor "Sir," and the letters should be signed "Yours faithfully." They should describe the observations within the compass of say 500 to 1,500 words, and make any necessary references to literature. They will then appear in a special section of our correspondence columns under the heading "Scientific Correspondence."

RETURN OF R.C.V.S. PRESIDENT

The stratocruiser bringing the President, Mr. G. N. Gould, home from his tour of the British Caribbean Federation and Venezuela was delayed by adverse weather conditions, so that he landed at midnight on Sunday evening, June 1st.

He reports that the tour was a great success and his reception by the Governors and Prime Ministers of the various territories was most cordial. In the final part of his tour in Trinidad he gave two papers before the British Caribbean Veterinary Convention, the first in the presence of the Minister of Agriculture. The exhausting round also included lectures and film demonstrations before a number of bodies, including the British Council, the Rotary Club, and the Turf Club, and on the radio. He was the guest of honour at a cocktail party given by the Government and had the pleasure of proposing the toast of the British Caribbean Veterinary Association at the 1958 Convention banquet.

Follicular Cystitis and Ureteritis with Renal Calculus in a Bitch

BY

J. E. T. JONES and R. CHURCHILL FROST

Royal Veterinary College and Hospital, London

SUMMARY.—A case of follicular cysto-ureteritis with renal calculus in a 9-year-old mongrel bitch is described.

THIS case is recorded on account of its unusual nature, its pathological interest, and because it was possible to diagnose renal and ureteric disorder in life.

Subject. Mongrel bitch aged 9 years. Weight 36 lb.

History. An intermittent vulval discharge during the 18 months preceding examination. There had been some treatment for nephritis.

Symptoms. The animal was in good bodily condition. The only evident abnormality was the presence of bloodstained shreds of mucus at the vulva.

Examination. Abdominal palpation proved negative but a radiograph of the abdomen in the lateral plane showed an abnormal shadow in the pelvis of one kidney. In view of this, urography was undertaken employing the technique described by Harrod and Frost (1955), using 12-c.c. radio-opaque material.* This clearly demonstrated the presence of a calculus in the left kidney and what appeared to be a well-defined dilatation of the left ureter. The right ureter appeared normal. Treatment was refused and at the express wish of the owner the dog was destroyed.

Post-mortem *Examination.* Pathological lesions were confined to the urinary system. The left kidney was of normal size, the capsule stripped easily, and the cut surface revealed a normal cortex and medulla. The pelvis was distended due to the presence of a sharp-edged calculus with a rough surface and measuring 3 × 1.5 × 1 cm. The pelvic mucosa was hyperaemic and studded with a few grey hemispherical nodules having a diameter of 0.5 mm. The ureter was dilated and in the upper third had a diameter of 0.5 cm. The entire length of the ureteric mucosa showed numerous well-defined glistening grey nodules of 1 to 1.5 mm. diameter. The right kidney and ureter were of normal appearance. The bladder was contracted and empty; its external surface was normal. The bladder mucosa was reddened and studded with many discrete nodules similar to those in the ureter but of a larger size, in some cases having a diameter of 2.5 mm. All had glistening surfaces, some were colourless and almost transparent, while a few were uniformly reddened. Some of the clear nodules were surrounded by a hyperaemic zone. Haemorrhagic, mulberry-like excrescences were present in the region of the fundus. There were no calculi in the ureter or bladder. The urethra was normal. The renal calculus consisted mainly of phosphates.

Histology. Section of the kidney showed evidence of pyelonephritis. The renal pelvis was heavily infiltrated with small round cells accompanied by a few fibroblasts. In the renal parenchyma circumscribed and diffuse areas of round-cell infiltration were evident. There was an early fibrous tissue reaction and some of the more distal parts of the tubules contained eosinophilic hyaline material.

The nodular lesions in the ureter and bladder consisted of unencapsulated dense aggregations of small round cells of the lymphocyte type. There were no germinal centres evident but otherwise they resembled true lymphoid follicles. The follicles were submucosal and in the smaller and presumably more recently developed ones, the overlying epithelium was intact but in the case of the larger follicles the epithelium was either very thin or absent.

Sections of the haemorrhagic excrescences showed a folding of the bladder epithelium giving the mucosa a cleft-like and villous appearance. Beneath the epithelium extensive areas of extravasation were evident.

In some areas the epithelium was absent. Many of the epithelial cells were vacuolated and in some instances the spaces were filled with eosinophilic hyaline bodies. A highly vascularised connective tissue had developed in the sub-mucosal region.

Comment. This case is of interest because in addition to the diagnosis during life of nephrolithiasis it was possible to demonstrate dilatation of the ureter by the administration of radio-opaque material. Further, the follicular lesions described are an uncommon *post-mortem* finding in the dog.

Follicular lesions of the urinary tract of the dog are described by Bloom (1954) who states that they are rare in the pelvis and ureter but frequent in the bladder. Runnells (1954) states that the follicular form of cystitis is said to be frequent in the dog.

Greene and Feldman (1940) have reported a case of follicular cystitis in an adult bitch diagnosed during life by cystoscopy and confirmed at autopsy. The lesions assumed the form of collections of round cells resembling true lymphoid follicles.

This is the first time we have seen follicular lesions of the renal pelvis and ureter and in our experience follicular cystitis is not common, only 2 examples of the latter having been observed in the last 3 years: both were incidental *post-mortem* findings in dogs which had no previous history of urinary tract disease.

The cause of the condition is unknown. In the case described there was no indication from the histological picture of the cause of the lesions. No acid-fast organisms were observed in smears and no significant organisms were recovered on routine cultural examination of material from the follicular lesions.

* Hypaque: Bayer Products Limited.

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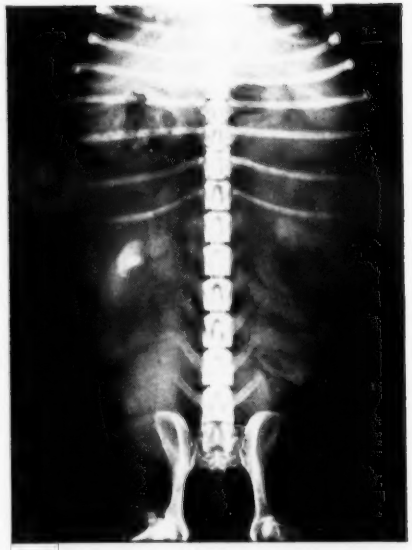

FIG. 1.

FIG. 2.

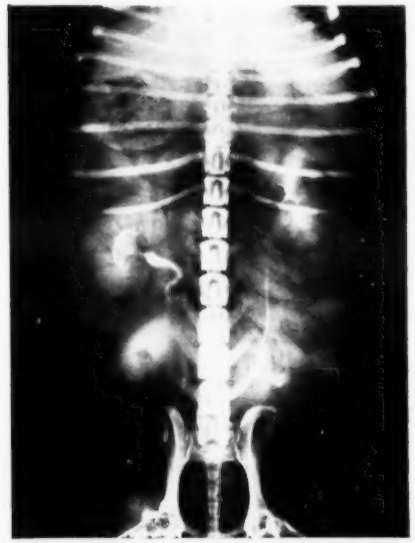

FIG. 3.

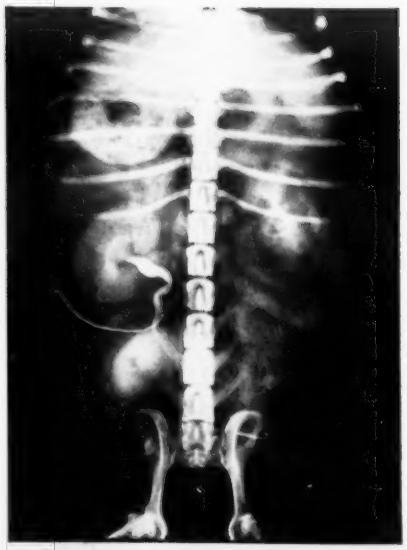

FIG. 4.

FIG. 5.

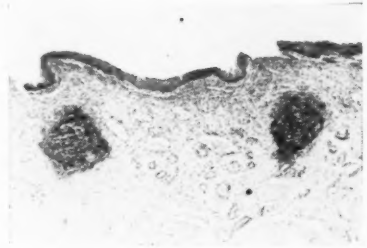

FIG. 6.

FIG. 1.—The control and before injection. The renal calculus can be seen.

FIG. 2.—Six minutes after commencement of the injection.

FIG. 3.—Twelve minutes after commencement of the injection.

FIG. 4.—Twenty minutes after commencement of the injection.

Figs. 2, 3, and 4 show various stages of excretion of the radio-opaque material. The calculus and the dilated right ureter are outlined. The normal left ureter is visible and some of the medium can be seen in the bladder.

FIG. 5.—Top left: normal kidney; top right: affected kidney, showing dilated pelvis with calculus *in situ* and the ureter with nodular lesions; bottom left: bladder, showing nodules and haemorrhagic excrescences; bottom right: renal calculus, showing its form.

FIG. 6.—Section of bladder wall showing two submucosal foci of round-cell aggregations. Haematoxylin and eosin. × 50.

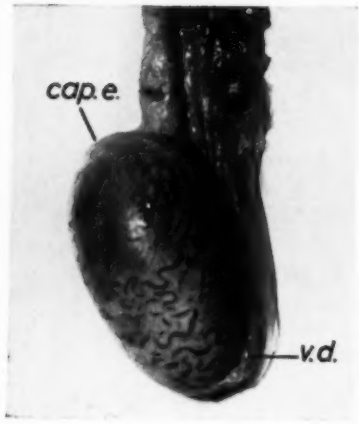

PLATE I.—Medial view of the right scrotal contents of a Friesian bull (Case 1) showing aplasia of epididymis and vas deferens. cap.e.: remnant of the caput epididymis; v.d.: cystic remnant of the vas deferens.

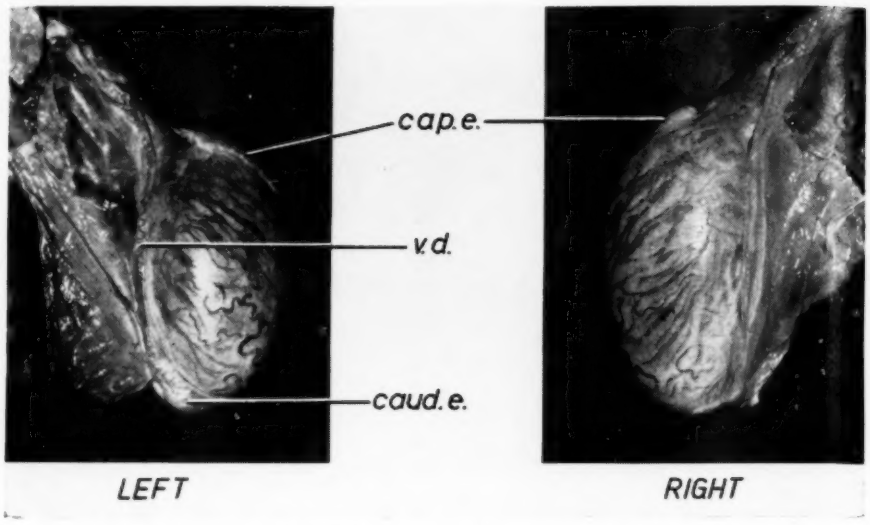

PLATE II.—Medial view of the scrotal contents of a Friesian bull (Case 2) showing aplasia of epididymis and vas deferens on the right side. The left side is normal. cap.e.: caput epididymis; caud.e.: cauda epididymis; v.d.: vas deferens.

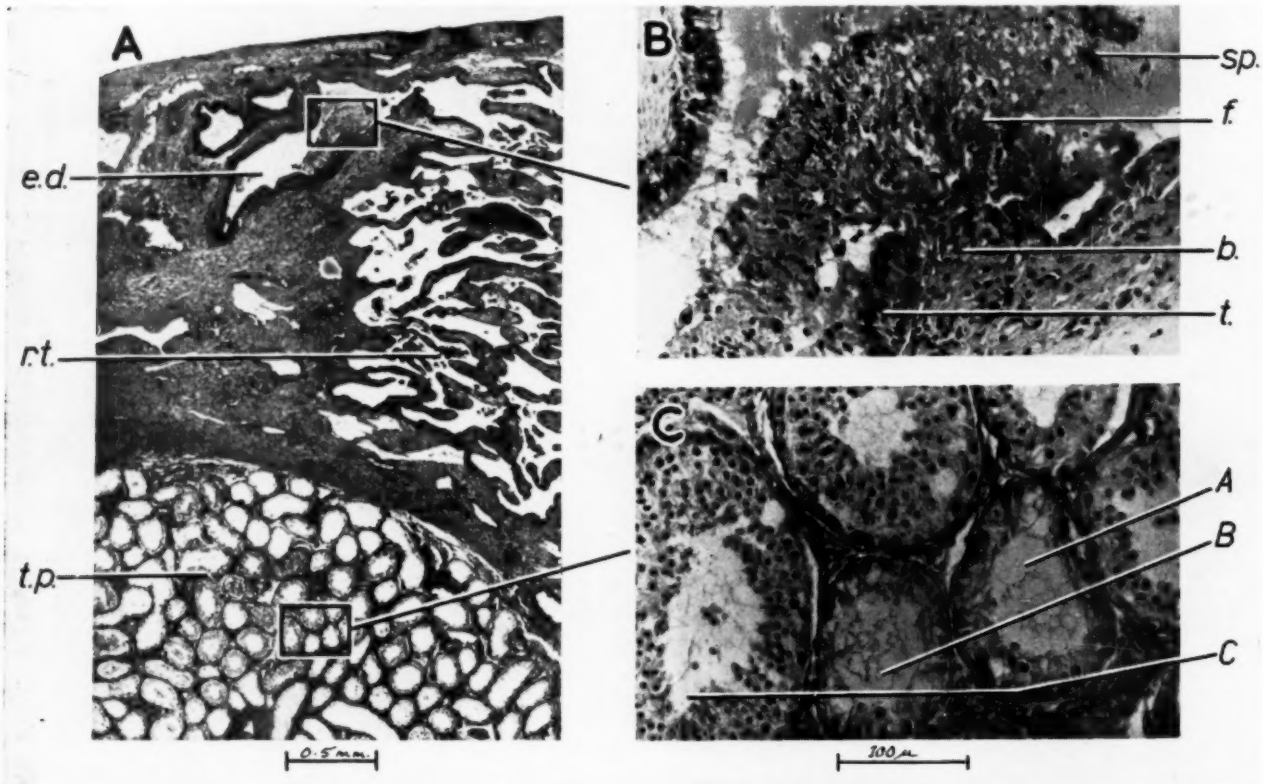

PLATE III.—Histological section of the right testis from a Friesian bull with aplasia of the epididymis (Case 1).

(a) Low power, showing testis parenchyma (t.p.), spaces of the rete testis (r.t.), and one efferent ductule (e.d.) at its origin from the rete testis.

(b) High power, showing the structure of an outgrowth projecting into the lumen of the efferent ductule. sp.: sperm clustered around the fringes of the outgrowth; f.: fibroblasts lying in the amorphous matrix; b.: base of the outgrowth, where the epithelium of the ductule is absent; t.: tunnels lined with epithelium lying at the base of the outgrowth.

(c) High power, showing two degenerate seminiferous tubules (A, B) adacent to one in which spermatogenesis is normal (C).

Aplasia Segmentalis Ductus Wolffii: a Report of Two Cases in British Friesian Bulls

BY

R. R. ASHDOWN

Department of Veterinary Anatomy, The University, Bristol, 8

SUMMARY.—The anatomical abnormalities found in two cases of segmental aplasia of the Wolffian ducts are described. One case was bilateral, affecting epididymides and vasa deferentia and causing aspermia of the ejaculate. In the other only the right epididymis, vas deferens, and ampulla were affected and the ejaculate was of good quality. All affected testes showed degenerative changes.

The condition is considered identical to that previously described in Denmark and the importance of clinical examination in diagnosing the disease is stressed in view of its probable hereditary nature.

Introduction

In a series of papers, Blom and Christensen (1947, 1951, 1952, 1956) have described an anatomical defect of the male reproductive tract in Danish bulls affecting the structures derived from the Wolffian, or mesonephric, duct (epididymis, vas deferens, ampulla, vesicular gland). In comprehensive surveys of adult bulls and calves of various ages they found incomplete development of these structures in about 0.5 to 1 per cent. of animals. In about 80 per cent. of cases the lesions were limited to the right side, and in only one case were they bilateral. The most common *ante-mortem* findings in adult bulls were: absence of the cauda epididymis; a marked tendency to retract the testis of the affected side; and abnormalities of the vesicular glands or ampullae on rectal palpation. Although the affected organs were usually absent or very small, occasionally they were enlarged by accumulation of secretory material in the occluded ducts. In uncomplicated unilateral cases fertility was often normal though sometimes, as might be expected, sperm counts were low.

Blom and Christensen found this defect in bulls of the following breeds: Red Danish Milk Race (1947); Black-and-White Jutland (1951); Friesland Friesians (1951); Danish Friesians (1956); but failed to find one case in 843 new-born Jersey calves. Two cases in Guernsey bulls have been reported from America (Gilman & Hopper, 1925; Roberts, 1956), and Van der Sluis (1953) has described the condition in Dutch bulls. In England, Rollinson (1950) recorded one case of absence of the epididymis in a Friesian, and Arthur (1956) recorded one in a yearling Shorthorn, but these authors gave no anatomical details of their cases. During the winter of 1956-7 two animals showing anatomical defects of the reproductive tract apparently identical to those described by the Danish workers were brought to my notice.

Materials and Methods

Both cases were found whilst investigating sexual development of bull calves at the Milk Marketing Board's calf-rearing unit at Chippenham. In this establishment pedigree bull calves are reared to about 18 months of age, semen is collected at intervals from bulls aged 9 to 12 months upwards, and behaviour and semen quality are recorded for each collection.

Both bulls were slaughtered at a Bristol abattoir and the genitalia were removed for further examination. The gross anatomy was recorded by measurement and photography. Specimens from the parenchyma of the testis, rete testis, epididymis, vas deferens, and seminal vesicles were taken for histological study. These were fixed in Bouin's fluid or formal saline, embedded in paraffin wax, sectioned at 7 or 10 μ, and stained by the following methods: H & E; Weigert's Haematoxylin and Van Gieson; Masson's trichrome. Spermatozoa from the epididymides were stained by H & E; Weigert's Haematoxylin; and crystal violet. The material from case 1 was fixed within 2 hours of death, but in case 2 there was an unavoidable delay of 24 hours, during which the genitalia were kept in the refrigerator.

Case Reports

Case 1

History and Ante-mortem *Examination*

This bull was the result of a son × dam mating. The rearing period had been satisfactory but as it reached puberty it showed a lack of masculinity. It would stand still for other calves to ride it and showed no desire to ride them or to masturbate. Nevertheless, from 12 to 19 months of age semen was collected on 10 occasions and each time libido and volume of the ejaculate were normal; the bull was usually keen to mount the teaser. The ejaculate was uniformly bad in quality—of the colour and density of urine and devoid of sperm.

On clinical examination no part of the epididymides or vasa deferentia could be palpated; both testes felt abnormally smooth and rounded at their dorsal and ventral poles. Rectal palpation revealed no abnormalities of the pelvic viscera. When 19 months old the bull was slaughtered as it was completely unfit for service.

Post-mortem *Examination*

The carcase was more like that of a castrate than an entire bull but the pelvic (prostate, vesicular glands, ampullae, bulbourethral glands, pelvic urethra) and external (scrotum, penis, sheath)

genitalia were apparently normal. A small Mullerian remnant persisted in the genital fold. The scrotal contents on the right were slightly heavier than those on the left (500 and 450 g.). Both testes were normal in size, shape, and consistency but there was an almost complete absence of epididymides and vasa deferentia (see Plate I). On both sides the sole remnant of the epididymis was a small, raised area about 2 cm. in diameter, at the dorsal pole. Typical contorted tubules were visible in this raised area. Cystic remnants of the vas deferens were present in the deferential fold of the mesorchium on both sides, but no sign of the ducts was found in either spermatic cord. Both of the ducts were present in the abdomen at their junctions with the two ampullae; they must, therefore, have commenced at about the level of the inguinal ring.

Puncture of the left epididymis released several cubic centimetres of a slightly cloudy fluid in which motile sperm were present at a concentration of about 200,000 per c.c. These were morphologically normal and about 95 per cent. showed proximal cytoplasmic droplets. The rete testis and the tubules of the small caput epididymis were markedly distended but the parenchyma of the testis appeared to be normal. Apart from an enlarged pancreas weighing 525 g. no abnormality was found in the rest of the viscera.

Histological Examination

The histology of the *seminiferous tubules* showed great variation, even between adjacent tubules. Some were normal, with active spermatogenesis leading to sperm production; in others, especially near the mediastinum, almost complete degeneration of the cells of the spermatogenic series had occurred and the tubules were lined with a single layer of Sertoli cells (see Plate III, a and c). Between these two extremes all gradations of degeneration could be seen. A syncytium of Sertoli cells occupied the lumens of some of the seminiferous tubules, while other tubules contained cells of the spermatogenic series and multinuclear spermiophagic cells. A few tubules were packed with masses of sperm; these tubules were always very degenerate and so were those which showed cystic dilatation. The *interstitial cells* showed no pathological change. Sperm were frequently seen embedded in the cuboidal epithelial cells lining the distended spaces of the *rete testis*. The spaces contained sperm, other cells of the spermatogenic series, and large, multinuclear, spermiophagic cells.

The tubules lying within the remnant of the epididymis were *ductuli efferentes;* no true ductus epididymis was seen. The ductuli efferentes communicated with the rete testis (see Plate III a). Their epithelial cells were irregular, ciliated, and contained eosinophilic secretory granules. The lumens of the efferent ductules contained cytoplasmic extrusions from their epithelial cells, sperm, spermiophagic cells, and short lengths of detached epithelial cells. A peculiar feature of the ductuli efferentes was seen; large outgrowths from the epithelium projected into their lumens at several points (see Plate III, a and b). The structure of these outgrowths was as follows: the epithelium was reflected over their basal portions but the main part of the outgrowths was devoid of an epithelial covering. "Tunnels" lined with epithelial cells were frequently seen at the base. The main mass was made up of an amorphous substance containing many pycnotic nuclei, and cells resembling fibroblasts radiated from the base into the mass of debris. Peripherally, the mass was infiltrated with sperm and large multinuclear spermiophagic cells were usually seen on the fringes. Frequently there was an infiltration of mononuclear cells (lymphocytes, plasma cells) in the subepithelial tissues immediately surrounding the basal stalk of the outgrowth.

The histology of the vesicular gland, ampullae, and vasa deferentia was normal.

Case 2

History and Ante-mortem *Examination*

On the sire's side this animal was derived from cattle imported from Holland in 1936, but there was no marked inbreeding in its pedigree. Rearing history was satisfactory and sexual behaviour had developed normally. At one year of age the first semen collection was made: mounting and thrusting were good and, though the first collection was of poor quality, on two subsequent occasions samples of good volume, density, and wave-motion were obtained. However, clinical examination at 13 months revealed the absence of the epididymis on the right side, exactly resembling the bilateral condition already described. There was no detectable asymmetry of the scrotum when viewed from behind and rectal examination failed to reveal any abnormalities. However, in view of the previous case and the findings of the Danish workers, slaughter was recommended.

Post-mortem *Examination*

The animal was 13 months old at slaughter and the carcase showed typical masculine conformation. In the pelvic genitalia the right vas deferens was absent except for a few millimetres that twisted round the blunt end of the ampulla. The ampulla was short and misshapen. No Mullerian remnant was seen. The external genitalia and the contents of the left scrotal pouch were normal, and the two scrotal contents were of approximately equal weight (left, 365 g.; right, 360 g.). The testes were approximately equal in size (left, 12 cm. × 7 cm.; right, 12.5 cm. × 7 cm.) but the right epididymis was lacking except for a small collection of tubules on the cranial pole of the testis (see Plate II). Two zones were distinguishable in the epididymis: an outer one in which the tubules were filled with a yellow, caseous material and an inner one from which sperm-containing fluid was withdrawn as in the previous case. Smears of the yellow material showed it to be composed of densely packed sperm. No trace of the right vas deferens was seen in the scrotal contents. On cutting the testes longitudinally the distension of the right rete testis was very plainly seen. Also, within the parenchyma of the right testis, especially near the mediastinum, small areas were found in which the seminiferous tubules were hard and yellow in colour.

Histological Examination

In this case a comparison was possible between affected and unaffected sides; the testis and epididymis were normal on the left side but on the right side the following changes were seen:—

There was degeneration of the *seminiferous tubules* similar to that already described in Case 1, but normal spermatogenesis was occurring in many tubules. In the small, hard areas near the mediastinum, degeneration was more advanced; sperm were tightly packed into the tubules, and fibrosis of the wall and early signs of calcification of the contents were visible.

The spaces of the *rete testis* contained many spermiophagic multinuclear cells, and the cuboidal epithelium contained many sperm embedded in its cytoplasm.

In the tubules of the *caput epididymis*, those of the outer zone were the true duct of the epididymis. They were normal in appearance with a regular, high columnar epithelium and packed with spermatozoa. In the inner zone vasa efferentia were found; owing to *post-mortem* change their mucosal surfaces were largely separated from the submucosa but the general structure was normal and, unlike Case 1, no outgrowths of the walls projected into the lumens.

The histology of the pelvic genitalia was not examined in this case.

Discussion

Examination of these two cases convinces me that the condition is identical to that described by Blom and Christensen (1951).

The histological changes within the testes, undoubtedly caused by the back-pressure built up within a closed yet active duct-system, correspond well with those described by the Danish workers in 1951. Oslund (1926) and Van Wagenen (1925) have shown that, although blockage of the vas deferens causes very little testicular degeneration, ligation of the efferent ductules in adult rats produces complete degeneration in a very short time. However, it seems that congenital occlusion of the efferent ductules in the bull does not cause such dramatic changes; probably the pressure that develops within such testes is less than in those ligated suddenly during active testicular functioning.

Blom and Christensen (1951) saw outgrowths into the spaces of the rete testis apparently identical to those which occurred in the efferent ductules of my first case. The origin and significance of these outgrowths is obscure. Their general appearance was that of an organising exudate projecting into the lumen of the ducts, with cells (especially sperm) trapped upon their fringes and at their bases signs of inflammatory reaction. Proliferative changes in the epithelium of the rete testis, seen by Blom and Christensen (1951), were not seen in these two cases.

Aplasia of the Wolffian ducts has received but little attention in the past from those interested in cattle-breeding in England. It rarely causes absolute sterility, and when only one side is affected the semen quality is often good, and the defect is only detectable by careful clinical examination. Perhaps it is because of this that it has not been more widely recognised. However, especially in view of the evidence of possible hereditary transmission put forward by the Danes (Blom & Christensen, 1951, 1956), some consideration should be given to the possibility of eliminating the defect from our breeding bulls. It is of considerable interest that a segmental aplasia affects the Mullerian (white heifer disease: Spriggs, 1946) as well as the Wolffian ducts of cattle, and in both cases there is room for further embryological research. However, there seems no genetic similarity between the two conditions and it is improbable that aplasia of the male ducts will ever be recognisable in the female, though the cow may carry the genetic defect.

Diagnosis of segmental aplasia of the Wolffian duct in the bull rests solely on the careful examination of the whole genital tract. When examining a bull for soundness, regardless of age or fertility, the veterinary surgeon should make certain that the epididymides, vesicular glands, and ampullae are normal. It must be stressed that some care is necessary before a definite diagnosis is made. Blom and Christensen (1951) observed that occasionally the cauda epididymis is medially displaced and more difficult to palpate than usual although normal in size. This condition also occurs in English bulls; once recognised it is fairly easy to palpate the epididymis of these animals after a little manipulation of the testes. This abnormal position of the cauda appears to be unrelated to aplasia of the epididymis.

Acknowledgments.—Messrs. B. M. Kerruish and R. S. Beynon brought these cases to my notice in the first place.

Messrs. B. M. Kerruish and J. P. Frappell collected all the data on early development and semen quality, and very kindly put their records at my disposal.

I must thank Professor C. W. Ottaway for his advice and encouragement. Also Miss P. Morgan and Mr. A. Coombs for technical assistance with histological preparations and photographs.

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Leptospirosis in Pigs*

BY

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LEPTOSPIRES are primarily parasites of rodents, chiefly rats, mice, and voles and are to be found in the tubules of the kidneys of these animals. The micro-organisms seem to exist as commensals without apparently producing any deleterious effect on the animal host. Intermittently some of the leptospires pass down the kidney tubules and are shed with the urine and if they find suitable wet conditions they can survive for days and even weeks. They enter new hosts probably through broken skin or mucous membrane and if these hosts are susceptible (man or domestic animal) they usually set up disease in them—*e.g. L. icterohaemorrhagiae* which is found very commonly in rats is the cause of Weil's disease in man; and the field vole is the reservoir host of *L. grippotyphosa* which is the cause of mud fever in man. *L. canicola* would appear to be an exception as the carrier host seems to be the dog.

The following serotypes have been associated with leptospirosis in swine: (1) *L. pomona* (*suis*), (2) *L. mitis* (*hyos*), (3) *L. icterohaemorrhagiae*, (4) *L. canicola*. It is proposed to deal with each species from the point of view of distribution, clinical symptoms in the pig, the effects on other animals, and lastly the public health significance.

L. pomona

L. pomona has been found in pigs in many parts of the world, *e.g.* Australia, parts of Asia and Europe, Argentina (*L. suis*), and is widespread in U.S.A., but has not been recovered from pigs in this country. Bryan (1957) stated that 20 per cent. of cattle and swine in the state of Illinois, showed evidence of infection. In recent weeks, pigs from one piggery in the City of Edinburgh have shown blood agglutination titres of 1:30 but it is too early to state the significance of this finding. Borg-Peterson and Fennestad (1956) found *L. pomona* in pigs to be absent from the mainland of Denmark, but present in the adjoining islands of Lolland and Falstar. In these islands the striped field mouse was found to show evidence of infection and since these rodents are not present on the mainland of Denmark it seems probable that they are the principal carrier hosts of *L. pomona* on the islands. This has been confirmed by reports from certain parts of Russia where field mice have also been shown to be responsible for harbouring *L. pomona* and passing the infection to cattle. However, it is generally considered that the pig is the main carrier host of this serotype with leptospiruria a very common sequel to infection.

Bohl *et al.* (1954) stated that in America clinical signs in the pig were very slight and transitory but in pregnant sows, if infected during pregnancy, abortion did occur frequently in the last 2 weeks of gestation with death of the new-born piglets. Sows which had aborted once had not been observed to do so again from this disease. Experimental infection of pigs with *L. pomona* resulted in a leptospiraemia of 4 to 6 days' duration with febrile reaction and inappetence for 1 to 2 days. The micro-organisms then became localised in the kidney tubules and were excreted in the urine for a variable period, up to one year. In Europe, inappetence and intestinal disturbances have been noticed in affected pigs and this may be followed by signs of nervous derangement, *e.g.* constantly moving in a circle, and an inability to stand on their legs (Van Thiel, 1948).

In the U.S.A. Ferguson and Bohl (1953) stated that *L. pomona* infection was also widespread in cattle. Clinical signs varied from an inapparent infection in some animals to an acute, rapidly fatal disease with marked haemoglobinuria in others. In their opinion atypical mastitis and abortion were probably more common from this infection than had previously been recognised. Bryan (1957) stated that morbidity approached 100 per cent. in a typical herd outbreak with a mortality of 5 per cent. or less. The losses occurred mainly in young stock. Cattle in Queensland and on the islands of Lolland and Falstar also show serological evidence of infection. Sutherland *et al.* (1949) described outbreaks in calves in Australia characterised by high mortality or in less severe cases by pyrexia, lethargy, hurried respiration, red urine, and jaundice. In the U.S.A., *L. pomona* has been incriminated in cases of ophthalmia in horses, and in acute cases severe jaundice with haemoglobinuria have been described.

From the public health aspect *L. pomona* was first isolated from cases of leptospirosis in dairy workers in the village of Pomona in Queensland in 1937 by Clayton *et al.* (1937). It was later found to be identical with the organism responsible for so-called "Swineherd's Disease" recorded by Gsell in Switzerland (1944). Derrick (1942) in Australia recorded 80 cases of human leptospirosis due to this serotype. Mihaljevic and Falisevac (1952) analysed 112 human cases of leptospirosis for the 1947-51 period in Yugoslavia, and found that 60 per cent. were due to *L. pomona*. Cases occurred in the summer and early autumn. Van Thiel (1948) quotes Gsell as stating that in Switzerland swineherd disease is also a summer disease and occurs in young swineherds. This was explained by the fact that the young employees were given the task of cleaning out the pig pens and in summer they wore no shoes.

L. mitis seems to be less virulent than *L. pomona*, but it has been recovered from pigs in Queensland, Europe, and the Argentine and is the cause of a

* Paper presented to the Scottish Regional Group of the Association of Veterinary Teachers and Research Workers in Edinburgh on November 27th, 1957.

benign leptospirosis of man known as "Seven-Day Fever." In Yugoslavia 10.7 per cent. of the 112 human cases were due to this serotype (Mihaljevic & Falisevac, 1952).

L. icterohaemorrhagiae

The first record of the isolation of *L. icterohaemorrhagiae* from pigs is that of Klarenbeek and Winsser (1937) who described an outbreak of jaundice in young pigs and noted that spontaneous recovery occurred in some of the affected animals. Field and Sellers (1951) described similar findings and confirmed the spontaneous recovery by experimental inoculations of piglets. Nisbet (1957) described 2 outbreaks in the South East of Scotland with clinical signs in small pigs of listlessness and jaundice, and with a high death rate. The infection has also been reported in Western Samoa where, following a serious outbreak of Weil's disease in the human population, investigations carried out by Johnson (1943) in Brisbane showed that infection was endemic in Samoan pigs.

In this country *L. icterohaemorrhagiae* infection was first isolated from dogs by Okell, Dalling, and Pugh (1925). Its presence in calves has also been recorded in England by Field (1949), Field and Sellers (1950), and by Ingram *et al.* (1952). Symptoms in calves as described by Ingram include emaciation with staring coat, listlessness, and jaundice to a varying degree, but there was no evidence of haemoglobinuria. Mortality rate even in jaundiced calves was low.

From the public health aspect this serotype is the most dangerous, giving rise to Weil's disease which, as is well known, is a very serious illness and in the period 1947-51 had a mortality rate of 15 per cent. in England and Wales (Broom, 1951). The same author analysed the occupations of the persons involved in the 418 cases of Weil's disease occurring in this period and found that 31 per cent. were agricultural workers. Four men out of a total of 13 infected in Edinburgh during the past 10 years were employed on farms with large piggeries where conditions may be particularly conducive to the survival and spread of the organism which is normally carried by rats.

L. canicola

L. canicola infection in pigs was first described in South Georgia by Williams *et al.* (1953) when human infection was caused by bathing in a creek to which dogs, cows and pigs had access and which were subsequently found to be infected. Three pigs showed positive agglutination titres and leptospires were recovered from the kidney of one. Van der Hoeden (1956) described 2 outbreaks in swine in Israel in which there were no apparent clinical signs in the first outbreak but in the second there were fever, anorexia, and convulsions, although the picture was complicated by the presence of *Salmonella suipestifer* in the piggery. On *post-mortem*, nephritis was shown to be present in affected pigs in both outbreaks. The leptospires were not recovered and the diagnosis was made on strong serological evidence alone.

Findings in Edinburgh (Coghlan, Norval & Seiler) in 1956 showed that 2 pig herds in the City were heavily infected with this serotype. In the first piggery 61 per cent. of 75 pigs and in the second 40 per cent. of 98 pigs tested showed agglutination titres of over 1 in 100. In addition, subsequent tests have shown that 8 other piggeries, within the city boundary, were infected as well as 2 in the adjoining county of West Lothian. All these pig farmers feed their animals on swill and damp conditions prevail in the pens and yards. No clinical symptoms have been observed in any of the pigs in the infected herds although, as a result of the common practice of overcrowding the pigs for heat retention, there are usually one or two slightly unthrifty animals in each pen. Testing of these pigs did not reveal higher agglutination titres than the bigger ones in the pen. There is no history of abortion in any of the herds. *L. canicola* was successfully recovered from the urine of naturally infected pigs by culture and inoculation of hamsters. Experimentally it was found that pigs were very susceptible both to infected pig urine and also to a virulent canine strain. The animals were infected successfully by inoculation subcutaneously, orally, and by skin scarification. The pigs showed a febrile reaction of approximately 24 hours' duration 7 days after inoculation and but for a slight lethargy showed no other symptoms. There was, however, a marked serological response and leptospires were recovered from the kidneys at autopsy. When young pigs were introduced into an infected farm, blood agglutination titres rose from less than 1 in 10 to 1 in 1,000 in one pig and in two others from less than 1 in 10 to 1 in 10,000 by the 8th week and leptospires were seen in large numbers in the urine and isolated from it by culture and hamster inoculation.

Various surveys carried out in this country have shown that 20 to 40 per cent. of dogs show serological evidence of infection with *L. canicola*. A recent survey by Cunningham *et al.* (1957) showed that 28.9 per cent. of 197 dogs tested in Glasgow were serologically positive and the authors remarked that in this urban area, despite the widespread use of penicillin, the incidence of infection has apparently changed little in the past 10 years although the mortality rate has probably dropped. It has been estimated that 25 per cent. of dogs in the United States have or have had leptospirosis and 25 to 50 per cent. of these become temporary carriers.

In Israel, Van der Hoeden (1955) found serological evidence to show that an average of 46 per cent. of dogs were positive. In the southern part of Israel, however, he considered jackals to be the main host of the serotype. He also described outbreaks of *L. canicola* infection in 4 herds of cattle. The majority of the cattle revealed no symptoms, but a few showed a fever of short duration, lack of appetite, jaundice, marked haemoglobinurea, and decreased milk yield. Experimental inoculation of a canine strain into the conjunctival sac of a calf induced an interstitial nephritis with leptospires excreted in the urine.

From the public health aspect *L. canicola* does not give rise to as serious an illness in man as *L. icterohaemorrhagiae* and the disease is rarely fatal. The

illness does, however, incapacitate the individual for several weeks and usually requires hospital treatment. Broom (1951) stated that in his experience persons who have been responsible for the nursing of a sick dog and cleaning up its dejecta have contracted the disease and that multiple cases in the household have occasionally occurred when the above duties were shared. In other cases, however, no connexion between patient and dog could be found. McIntyre and Seiler (1953) found, however, that not one of 99 persons in the City of Edinburgh who had contact with dogs suffering from a *L. canicola* infection showed any serological evidence of being infected with this serotype. A similar report from Austria shows that only 2 human cases occurred among the owners of 7,370 similarly infected dogs (1953 W.H.O. Monograph, No. 19, page 123).

In 1956, on the other hand, we found that 40 per cent. of blood samples taken from 47 piggery workers showed agglutination titres to *L. canicola* in amounts sufficient to signify a past infection with this serotype. Included in this number were 5 clinical cases of canicola fever and 5 whose history of illness suggested a similar infection. During the past 12 months a further 11 human blood samples from suspected cases of canicola fever have been found to be positive. One of the affected persons is employed as a pig slaughterman at the local abattoir and 7 others had worked in or had access to piggeries. The last case reported—a 6-year-old girl—had been playing in an infected piggery a fortnight prior to the onset of symptoms. In Israel, Van der Hoeden found that in the piggeries concerned with the 2 outbreaks which he described, 5 piggery workers in one, and 3 workers in the other, showed serological evidence of infection (Van der Hoeden, 1956).

Various theories may be advanced to explain the higher infection rate in piggery workers compared with dog handlers:—

1. Dog handlers will tend to wash their hands after being in contact with infected urine, whereas piggery workers may not do so.
2. If a large pen of pigs is infected the number of leptospires excreted must be very great.
3. On a carbohydrate diet the urine of pigs is less acid than dogs, with the result that the micro-organisms will survive longer.
4. Wet conditions tend to allow the organisms to survive longer and to be distributed readily among non-infected pigs.

Prevention

As *L. pomona* has been proved to cause quite heavy losses in livestock in America a great deal of research work has been carried on in recent years to control that disease. York and Baker (1953), Bryan (1957), and Brunstein *et al.* (1957) have recorded successful control in both cattle and pigs with the use of a dead vaccine. York (1957) stated that cattle with as low an antibody titre as 1:10 to 1:40 withstood a challenge from a virulent strain, capable of causing illness in control animals. He found that certain strains of the serotype had greater antigenic producing qualities than others. After vaccination immunity is considered to last 18 months. He estimated that 1,500,000 cattle and swine have been vaccinated in the past 2½ years in the U.S.A. Attempts have also been made to eliminate this infection by the use of antibiotics, and Baker *et al.* (1957) recorded the successful elimination of *L. pomona* from carrier swine by feeding terramycin for 7 days at the level of 500 to 1,000 grammes per ton of food.

In this country *L. canicola* infection in pigs does not seem to present any immediate economic problems, but if it is found that infection of piggery workers in other parts of the country is as high as in Edinburgh, then there is no doubt that this infection is of considerable public health importance. At present reliance must be placed on fairly strict hygienic measures. Piggeries should be kept as dry as possible and hypochlorite disinfectants should be used regularly. After the vacation of pens (by fat pigs) they should be thoroughly disinfected before they are again occupied by fresh pigs. Rubber boots in good repair should be worn by the workers and cuts on hands should be properly covered with a surgical dressing. In view of the serious danger to pigs and man of *L. icterohaemorrhagiae* infection, rats should be eliminated as far as possible by poisoning. Dogs should not be kept for the purpose of rat control and, in view of the fact that they may introduce *L. canicola* into the piggery, it would seem advisable to ban them from piggeries completely.

Acknowledgment.—I am greatly indebted to Dr. J. C. Coghlan, PH.D., B.SC., for her helpful advice and criticisms in the preparation of this paper.

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(*Concluded at the foot of col.* 1, *page* 484)

The Influence of Storage Conditions for Blood Samples on the Analysis of Serum

BY

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SUMMARY.—1. *During a delay of a few days in the determination of certain serum constituents, considerable changes occurred in their levels during storage. Consequently experiments were carried out to establish storage conditions under which the level of the serum constituents investigated could be maintained over a suitable period.*

2. *The changes in the levels of the following constituents of bovine serum during storage were determined, using the blood of normal cows: glucose, citrate, pyruvate, α-ketoglutarate, ammonia, urea, amino-nitrogen, and cholesterol. Recovery experiments with ketone bodies which had been added to the serum, were also carried out. Some of the orthodox methods used had to be considerably modified to suit the purpose.*

3. *Though the addition of sodium fluoride to blood samples ensured the stability of some constituents for a considerable time when the samples had been exposed to ambient temperature, other constituents, such as ketone bodies, proved to be much less stable unless the samples were kept in a frozen state.*

4. *Preliminary bacteriological investigations showed that the rapid breakdown of urea in the serum in the absence of fluoride was due to bacterial infection, but not to the activity of* Proteus vulgaris, *a common bovine skin contaminant.*

5. *The findings with regard to storage conditions for the individual serum constituents are listed.*

Introduction

DURING studies of bovine ketosis the composition of bovine serum in normal and pathological animals was examined, particularly with regard to components which hitherto had received only little attention. Comparatively little is known, for instance, of the levels of serum constituents such as pyruvate, α-ketoglutarate, and citrate. Moreover, generally little information has been given in the past as to the conditions for the storage of the serum samples (Braun, 1946; Hewitt, 1930; Reihart, 1939; Shope, 1928; Teeri *et al.*, 1946). Yet a clear definition of the storage conditions appears to be particularly important, for it was found during the initial stages of this work that the variations observed in the individual samples taken from normal animals were to a large extent due to insufficiently controlled storage conditions for the serum. It was seen, for instance, that pyruvic acid accumulated in a short time during standing in the refrigerator while the levels of other components decreased; very few serum constituents could be relied upon to stay at a constant level even for the 24 to 48 hours which usually passes from the time the blood sample is taken until it is analysed. It was, therefore, decided to study the changes in the levels of 11 serum constituents during standing under various conditions and to elaborate suitable analytical methods.

Methods

Animals. Normal dairy cattle of the University Field Station provided the blood used in all investigations except in the experiments on cholesterol levels (Table V) where the blood was collected from the abattoir.

Collection of samples. Two oz. of blood were collected in two 1-oz. screw-capped bottles. When NaF was used, 2 ml. of a 1 per cent. solution were added to the bottle which was subsequently heated in an oven at 110° so that 20 mg. of the salt were deposited as a thin layer on the inside. The clotting of the blood was not affected by this concentration of fluoride. The blood was taken from the jugular vein of the animals and was allowed to flow for a few seconds before collection. Then the bottles were filled and gently shaken, leaving only a small air space at the top. Abattoir samples were collected after stunning and during the process of bleeding. In all cases the blood was kept at room temperature for 12 to 24 hours after collection for clotting, after which the serum was separated by centrifugation of the bottles, decanted, and then stored in closed containers under the various conditions described in this work until the samples were analysed.

Estimation of glucose. The Fujita and Iwataki (1931) modification of the Hagedorn and Jensen method was used as described by Baldwin and Bell (1955).

Estimation of pyruvate and α-ketoglutarate. Hydrazones of these compounds were prepared according to Cavallini and Frontali (1954). The hydrazones were then placed on strips of Whatman 3-mm. paper (as used by El Hawary and Thompson, 1953) by means of a micrometer syringe. The syringe was fitted with a glass needle and was operated by a motor fitted with a double-worm reduction gear. By using the micrometer syringe it was possible to obtain spots for paper chromatography of less than 0.6 cm. diameter from 0.2 ml. of fluid. The spots were dried with a hair dryer which was adjusted for position so that the temperature of the spot was maintained at approximately 60°. At this temperature any acetoacetic acid hydrazone present, which would interfere with the method, is converted to acetone hydrazone which, according to McArdle (1957), does not interfere. After equilibration of the spotted paper for 18 hours the hydrazones were separated by descending chromatography over a 30-hour period using a mixture of

tertiary amyl alcohol, ethanol, and water (50/10/40 by volume) as the solvent system (Altmann, Crook & Datta, 1951). The single α-ketoglutarate spot and the double pyruvate spots were cut out after being outlined with pencil under ultra-violet light. After extraction of the spots according to the method of Cavallini and Frontali, the optical density was measured at 4,650 A° in a Hilger absorptiometer using 1-cm. cells. The values were read from calibration graphs for the two keto acids. The method is unaffected by NaF at the concentration used in this work.

Estimation of citric acid. The pentabromacetone method of Taylor (1953) was used, subject to considerable modifications. These modifications were necessary to adapt the method for serum, and to eliminate the interference of serum-ketone bodies as reported by Puncher, Sherman, and Vickery (1936) and also by Weil-Malherbe and Bone (1949). The interference by ketone bodies was confirmed by recovery experiments in this work. A further source of inaccuracy, as reported by Weil-Malherbe and Bone (1949), namely the contamination with acid of the petroleum ether extract of pentabromacetone was also eliminated as described below.

The method used was as follows: to 7 ml. of 25 per cent. trichloracetic acid in a centrifuge tube 5 ml. of serum were added. The mixture was shaken, allowed to stand for 10 minutes, and centrifuged for 10 minutes; 10 ml. of the deproteinised serum and 2 ml. of 27N sulphuric acid were pipetted into a glass-stoppered tube. The mixture was boiled for 3 minutes to remove acetoacetic acid and acetone quantitatively as shown by separate experiments. After cooling, the following additions were made: 8 ml. of 27N sulphuric acid, 2 ml. water, and 10 ml. of bromide-bromate-vanadate solution (Taylor, 1953). The mixture was shaken and allowed to stand for 30 minutes. Then 4 ml. of 22 per cent. ferrous sulphate solution were added and the mixture was again shaken at frequent intervals for 10 minutes to remove all traces of bromine. To the green solution 6.5 ml. of petroleum ether (80°–100°) were added and the mixture was once more shaken continuously for 1 minute. The lower aqueous layer was sucked off and traces of acid were removed from the petroleum ether layer by shaking it with 2 ml. of N/1 sodium carbonate solution. The aqueous layer was again removed and the petroleum ether extract was finally dried with a little anhydrous sodium sulphate. To 5 ml. of the petroleum ether extract, 3 ml. of freshly prepared 2 per cent. sodium sulphide solution were added. The mixture was shaken for 1 minute during which a yellow colour developed in the aqueous layer. After 10 minutes' standing in subdued light to retard fading, the aqueous layer was filtered through a dry Whatmann No. 1 filter paper, and the colour intensity was measured 15 to 30 minutes after the addition of the sodium sulphide in a Hilger absorptiometer at 4,300 A°. The citric acid values were obtained from a calibration graph. In recovery experiments with serum containing ketone bodies and known amounts of citric acid, the experimental error did not exceed 5 per cent.

Estimation of amino-nitrogen. The method of Frame, Russell, and Wilhelmi (1943) with the modifications of Russell (1944) was used.

Estimation of urea and ammonia. Urea and ammonia were determined by the method of Conway (1947). In the case of urea the modifications developed by Baldwin and Bell (1955) were adopted.

Estimation of Ketone Bodies. Ketone bodies were estimated by the method of Thin and Robertson (1952).

Estimation of Cholesterol. Total cholesterol was determined by the method of Sackett (1925).

Bacteriological procedures. For the preparation of sterile serum a Seitz filter was used. For the inoculation of serum samples with *Proteus vulgaris* sterile 5-ml. samples of serum with and without NaF were inoculated with one drop of a suspension of *Proteus vulgaris*. The suspension used was a one-in-250 dilution of an overnight culture in a nutrient broth. *Proteus vulgaris* counts on the serum samples were made on MacConkey's bile-lactose-agar medium using the technique described by Miles and Misra (1938). A count of the viable bacteria present in the serum samples which were collected under normal field conditions was made by the plate method using nutrient agar as the medium.

Results

Stability of Nitrogenous Constituents of Serum

Ammonia and Urea

Ten serum samples were investigated for changes in ammonia and urea content during several days' standing at room temperature. From the results of a typical experiment shown in Fig. IA it can be seen that in the absence of added NaF the ammonia level rose rapidly from about 1 mg. per cent. ammonia nitrogen to approximately 7 mg. per cent. in a few days. This rise was accompanied by a fall in urea nitrogen, though urea disappeared to a lesser extent than ammonia was formed. Thus, the ammonia must have originated partly from nitrogenous sources other than urea. This is further borne out by experiments with NaF: the presence of NaF stabilised the urea level completely but did not prevent a small rise in ammonia.

The effect of bacterial contamination. That the changes seen in Fig. 1A were due to bacterial contamination is seen from experiments with sterilised serum (Fig. 2A) where no changes in the levels of urea and ammonia were observed during standing whether or not NaF was present. On the other hand, in the contaminated serum the changes in the levels

TABLE I
CHANGES IN THE UREA AND AMMONIA CONTENT OF SERUM AND IN BACTERIAL COUNT DURING STORAGE

	Increase in ammonia, per cent.	Decrease in urea, per cent.	Increase in bacterial count ml. serum
Without NaF	550	13·5	200,000,000
With NaF ...	150	3·0	5,000

The serum was left standing in closed bottles for 7 days at room temperature.

Fig 1.

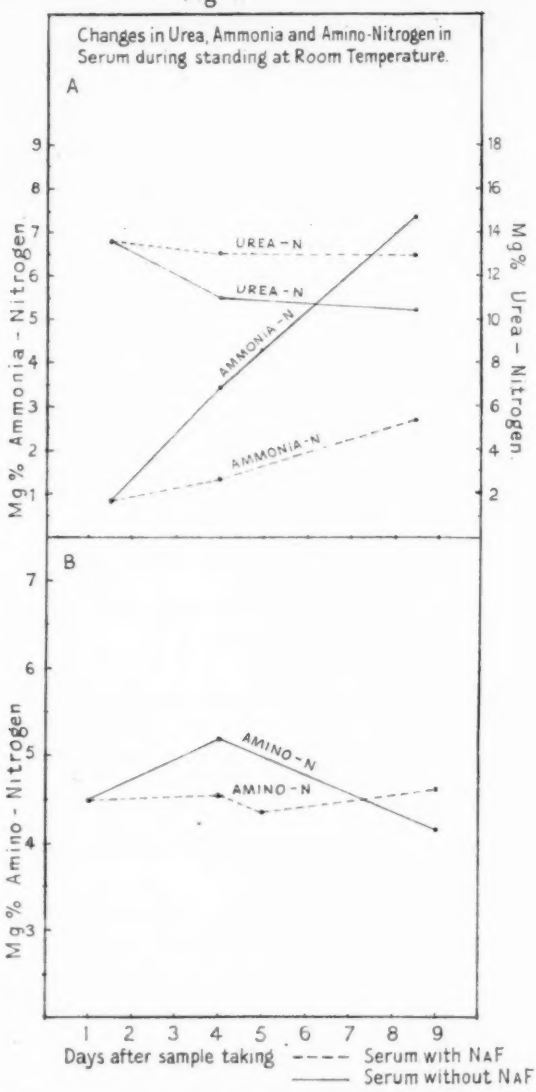
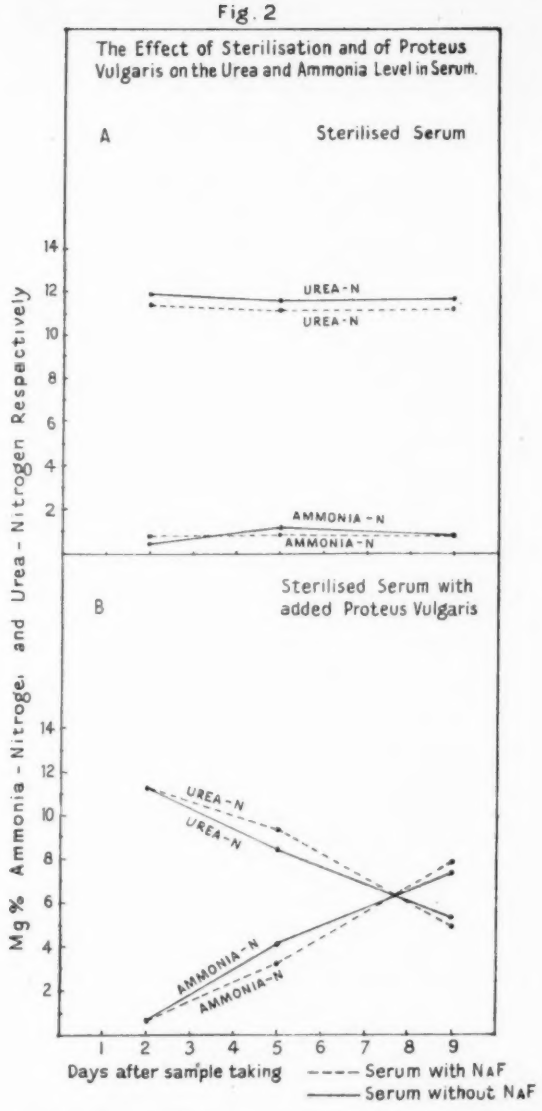


Fig. 2



of urea and ammonia were accompanied by an increased bacterial count (Table I). When NaF was added to the contaminated serum the changes in the bacterial count and in the levels of the above components were much smaller.

It was thought that *Proteus vulgaris*, a common bovine skin contaminant, which is known to contain urease, may have been mainly responsible for the above changes in urea and ammonia. However, experiments with this organism showed that this was not the case. Sterile serum was inoculated with *Proteus vulgaris* and was left standing for a period of 9 days. During this period the urea level dropped at the same rate as the ammonia level rose (Fig. 2B). These changes appear therefore to be solely due to the urease action of the organism. No such deduction can be made from the experiments with "naturally" contaminated serum (Fig. 1A) where the rise in ammonia did not correspond to the drop in urea. Furthermore, while the action of the "natural" contaminants could be inhibited by NaF (Table I), the latter had no effect on the levels of either of the nitrogenous components in the *Proteus*-inoculated serum (Fig. 2B). Nor was the count of *Proteus vulgaris* in the latter affected by NaF (not recorded).

These observations prove that the changes observed with contaminated serum cannot to any great extent be attributed to the action of *Proteus vulgaris*.

Effect of temperature. The effect of temperature on the formation of ammonia in serum during standing is shown in Table II. When NaF had been added, the samples were stable at room temperature for

TABLE II
THE EFFECT OF STORAGE TEMPERATURE AND SODIUM FLUORIDE ON THE AMMONIA LEVEL OF SERUM (MG. PER CENT. AMMONIA-NITROGEN)

Days after sample taki g	18°		−15°	
	With NaF	Without NaF	With NaF	Without NaF
1	0·98	1·13	0·98	1·13
3	0·98	1·68	0·98	1·68
6	1·68	3·08	0·84	1·96
9	3·65	6·45	1·26	2·10

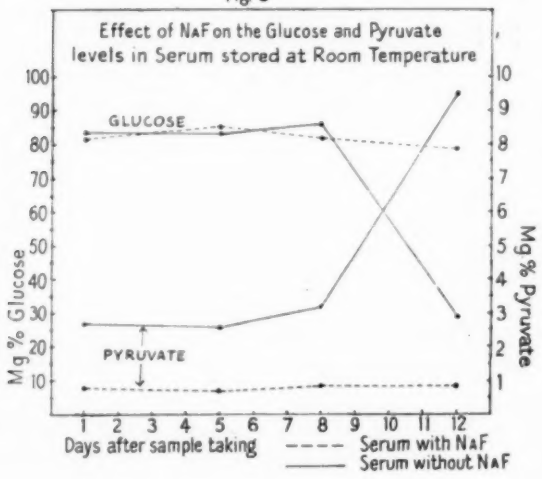
three days. After 3 days, stability could only be maintained when the samples were stored at −15° in the presence of NaF. Even freezing could not prevent decomposition after the elapse of 6 days.

Amino-nitrogen. As seen in Fig. 1B, changes in amino-nitrogen during standing at room temperature were negligible when NaF had been added to the sample. In the absence of NaF there appears to be a definite increase in amino-nitrogen on the 4th day, followed by a drop of amino-nitrogen between the 4th and the 9th day. This could be explained by bacterial action on the serum-proteins which would eventually lead to a liberation of ammonia, possibly preceded by the formation of free amino acids. Such a view is compatible with the results of Fig. 1A from which it was deduced that the rise in ammonia during standing was not solely due to the breakdown of urea, but must have been caused by bacterial action on other nitrogenous compounds, such as proteins.

Stability of Glucose and Pyruvate in Serum

Fig. 3 clearly demonstrates a relationship between the above two components. The level of glucose dropped at room temperature during the standing period which included the clotting time, at approximately the same rate at which the pyruvate level increased. Because of the rapid further metabolism of pyruvate a strict stoichometric relationship between glucose disappearance and pyruvate formation could not be expected. This experiment was repeated several times with the same result. It was also shown

Fig. 3



that in all cases the addition of NaF stabilised both the glucose and pyruvate levels completely, even when standing for 12 days at room temperature. In the absence of NaF refrigeration of the serum samples at −15° slowed down the process of fermentation, but could not fully prevent it (not shown in Fig. 3). It was further observed that in the absence of NaF the initial pyruvate level had risen during the one-day-clotting period to a value well above the normal. Since there was no corresponding fall in the glucose level, the rise in pyruvate remains unexplained. Separate recovery experiments have shown that the addition of NaF to the serum does not interfere with the pyruvate estimation (see Methods), therefore the initial pyruvate level in the presence of NaF must be regarded normal.

Stability of α-ketoglutarate

From the results shown in Table III it is evident that in the presence of NaF the α-ketoglutarate of the serum remains stable at room temperature for at least 5 days after the collection of the sample. In the absence of NaF its level drops rapidly to almost zero.

TABLE III
THE EFFECT OF SODIUM FLUORIDE AND STORAGE TEMPERATURE ON THE LEVEL OF α-KETOGLUTARATE IN SERUM (MG. PER CENT.)

Storage temperature	18° (sample 1)		−15° (sample 2)
Days after sample taking	Without NaF	With NaF	With NaF
1	0·31	0·31	0·14
3	0·27	0·28	—
4	0·14	0·28	0·13
5	0·16	0·33	—
6	0·06	0·32	—
7	—	—	0·15

Stability of Ketone Bodies

Since the level of ketone bodies constitutes a principal characteristic in certain pathological conditions, it was of importance to investigate the stability of these compounds in the first few days after sampling during which period the sample may be on its way to the investigating laboratory.

It was thought that losses in acetone could occur when the bottles are opened for sample taking, though care was taken to leave only a small air space over the liquid level so as to avoid accumulation of acteone. In Table IV the changes at room temperature in the acetone level of the serum stored in ordinary metal-capped bottles are compared with those stored in rubber-capped bottles (from which samples can be withdrawn with a syringe without opening). It will be seen that up to a storage time of 4 days no significant loss in acetone was incurred with either type of bottle. However, with longer storage periods a decrease in acetone was observed when metal-capped bottles were used, though not with rubber-capped bottles. No significant losses of acetoacetate and β-hydroxybutyrate were found during the first 5 days at 18° (Table IV). When the period was prolonged, the levels of these components dropped considerably. A storage tem-

TABLE IV
STABILITY OF KETONE BODIES ADDED TO NORMAL SERUM IN THE PRESENCE OF SODIUM FLUORIDE

Days after sample taking	Acetone content of serum stored at 18° in			
	Rubber-capped bottles		Metal-capped bottles	
	Mg. per cent.	Percentage loss	Mg. per cent.	Percentage loss
1	50·8	—	48·5	—
2	51·2	0	49·5	0
3	51·5	0	52·0	0
4	50·2	1·0	47·0	3·0
5	50·4	1·0	45·3	6·5
6	49·1	3·5	46·2	5·0
7	50·4	1·0	42·8	11·5
	Percentage loss in*			
	Acetoacetic acid		β-Hydroxybutyric acid	
	18°	−15°	18°	−15°
5	0	0	0	6
8	79	21	12	6

* Stored in metal-capped bottles.

perature of −15° appeared to be of some stabilising value although even in the frozen state appreciable losses in acetoacetate were observed after 8 days. When such experiments were carried out with rubber-capped bottles, the loss of acetoacetate was accompanied by a corresponding rise in acetone which implied a conversion of the former to the latter by decarboxylation (not recorded).

Stability of Citric Acid in Serum

As seen from Table V the citric acid level was reasonably stable when stored at room temperature for 2 to 3 days and completely stable even for longer periods when the serum was kept in the presence of NaF at 4°.

Stability of Cholesterol

After the estimation of their cholesterol content, 4 serum samples were stored, each at 18° as well as at 4° (Table V). The initial concentrations varied well within the normal range (50 to 230 mg. per cent, Dukes, 1955). The changes observed during a storage period of 4 to 6 days can be considered to be within the limits of experimental error. There was no indication that lower storage temperatures increased the stability of the cholesterol component, nor could any definite effect of NaF be observed.

Discussion

Although known methods were used for the analysis of the 11 components investigated in this work, some of the procedures had to be considerably modified to render them applicable for serum investigations. From the results obtained, it is evident that when serum is exposed in absence of NaF to room temperature or to temperatures prevailing during its transport to the investigating laboratory, considerable changes in the levels of its constituents occur within a short time. In the presence of NaF the period during which constancy of serum constituents could be maintained ranged from 3 to 12 days, but was never less than 3 days even at room temperature. A period of 3 days would only be sufficient for the transport of the blood sample, for the clotting time, and perhaps for the determination of one or two components; more time would have to be allowed if several components had to be estimated. For storage periods up to approximately 8 days the addition of NaF to the blood sample was sufficient to maintain the stability at room temperature of most constituents, but not of ammonia and the ketone bodies; to prevent a change in the latter component the sample had to be kept in a frozen state.

A few preliminary experiments were carried out on the nature of the contaminating organisms responsible for the rapid breakdown of urea to ammonia in the serum samples. The decomposition of urea was found not to be due to *Proteus vulgaris*, although it was evident that the decrease in the urea level and the slightly greater increase in that of ammonia during storage in "naturally" contaminated serum was due to bacterial action of some kind. As anticipated, a relationship was found between the bacterial count on the one hand and the degree of decomposition on the other, since both were reduced by NaF. Storage of the serum in a frozen state and in the presence of NaF prevented ammonia formation altogether, and this only for a period not exceeding 6 days.

The level of glucose was surprisingly stable at room temperature for 8 days, after which in the absence of NaF it fell rapidly and was accompanied by a rise in pyruvate. In view of the metabolic relationship between pyruvate and glucose during glycolysis this was to be expected. Both the pyruvate and the glucose levels were completely stabilised by NaF up to 12 days even when the serum was stored at room temperature. NaF was equally effective in stabilising the level of α-ketoglutarate for at least 5 days. The levels of acetone, acetoacetate, and β-hydroxybutyrate as well as that of cholesterol

TABLE V
STABILITY OF THE LEVELS OF CITRIC ACID AND CHOLESTEROL IN SERUM IN THE PRESENCE OF SODIUM FLUORIDE (MG. PER CENT.)

Days after sample taking	*Citric Acid* Storage temperature				
	18°	4°			
1	4·14	4·66	4·60	4·45	5·15
2	—	4·66	4·40	4·60	5·00
3	3·95	4·76	4·53	4·38	5·15
4	—	4·72	4·58	4·32	5·17
5	—	4·76	4·66	4·38	5·05
6	—	4·66	4·53	4·38	5·00
7	3·84	4·73	4·53	4·30	4·97
	Cholesterol				
1	172 (172)	136	146	192	172 (172)
4	168 (175)	141	139	200	161 (169)
6	165 (163)	138	135	189	169 (173)

Figures in parenthesis refer to serum samples in absence of NaF

could be maintained in the presence of NaF for 4 to 5 days. After that period serious losses occurred. Citric acid in serum can be stored even for longer periods without loss when the samples are kept in the refrigerator. The principal results with regard to storage conditions for the 11 serum constituents investigated are listed below:—

Serum constituent	Without NaF: Storage temperature	Without NaF: Stable for	With NaF: Storage temperature	With NaF: Stable for
				Days
Glucose	18–20°	8 days	18–20°	12
Pyruvate	18–20°	0 days	18–20°	12
α-ketoglutarate	18–20°	<3 days	18–20°	5
Citric acid	.....	.....	18–20°	3
	.....	.....	4°	7
Ammonia	18–20°	<1 day	18–20°	3
	−15°	<3 days	−15°	6
Amino-nitrogen	18–20°	2 days	18–20°	7
Urea	18–20	<2 days	18–20°	9
Acetone	.....	.....	18–20°	4
	.....	.....	−15°	9
Acetoacetic acid	.....	.....	18–20	5
	.....	.....	−15°	8
β-hydroxybutyric acid	.....	.....	18–20°	5
	.....	.....	−15°	8
Cholesterol	18–20°	6 days	18–20°	6

Acknowledgments.—We are indebted to Dr. A. H. Linton, Dept. of Bacteriology, University of Bristol, for carrying out the bacterial investigations, and to Mr. H. Pearson, Dept. of Veterinary Surgery, for providing blood samples from the University herd. We gratefully acknowledge the financial support for this work given by the Agricultural Research Council.

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Clostridial Enteritis in Cattle*

BY

G. N. SHIRLEY

Middlewich, Cheshire

IN presenting this short paper I do not wish to pose as an authority on the subject, or even to claim anything more than the amount of knowledge one inevitably acquires by frequent contact with the same condition. I do hope, however, that I shall gain some further information from any discussion which may be stimulated, and therefore I will simply report a series of facts as they have occurred.

As far as I know, the first occasion on which this disease appeared was some 10 to 12 years ago, when it appeared on a farm killing 7 cows in the course of 3 weeks, and altogether 14 cows in less than 6 months. I was not attending the farm at the time so I have no first-hand evidence of this particular case, but I do know the practitioner concerned sent samples to the laboratory for analysis as he was convinced that the condition was one of acute poisoning. I may say that all of us who saw any of these carcases at *post-mortem* examination immediately agreed that death was obviously due to poisoning. But all laboratory reports came back with a negative for poisons.

Over a period of 12 to 18 months isolated cases kept appearing at *post-mortem* examinations and I think it is true to say that most local practitioners had a "nibble" at the poison theory, sent samples to the laboratories and duly received negative reports. Equally negative were the reports from bacteriological examinations. Gradually we came to accept these occasional unexplained deaths as a minor nuisance which was not terribly important but which called for some considerable imaginative effort to explain satisfactorily to the particular farmer who had just lost his best cow!

Symptoms and *Post-mortem* Appearance

I think, at this stage, it would be as well to describe the actual disease. In the majority of cases no symptoms are observed—it is simply a case of sudden death. Again, in probably 80 to 90 per cent. of cases it occurs very shortly after calving—within the first 48 hours. In the less acute cases a condition closely resembling milk fever may occur. The cow is down and semi-conscious, but the condition differs from milk fever in that the temperature is usually raised and may be anything from 103° to 106° F. Oddly enough a calcium injection at this stage often produces a temporary improvement in the animal's general condition, but the improvement is short lived and death ensues fairly soon, preceded by a period of violent pain.

On *post-mortem* examination the fourth stomach shows marked inflammation of the lining. Acute enteritis is seen sometimes in only a portion of the bowels, but more often it extends throughout the

* Paper presented to the Lancashire Veterinary Association at its meeting on November 1st, 1957.

whole length of the intestines. This enteritis is diffuse and is usually so severe that the wall of the bowel resembles red velvet, with the bowel contents fluid and blood-stained or dark brown in colour.

Growth of the Disease in Cheshire

Returning to the development of the disease around our district, isolated cases were becoming slightly more frequent, and then some 6 or 7 years ago I met my first case of multiple infection. Ten animals were affected at once; 3 of them died in the usual rapid manner, 4 died after a short illness, and 3 animals survived but were left such complete wrecks that they were useless. I called in Mr. Francis (who was then with I.C.I.), to advise on this outbreak, but we still did not get to the bottom of it. Oddly enough this farm has never had another case since then.

More and more isolated cases were now appearing, and the condition was obviously becoming serious. We had for some time reached the conclusion that the causal agent must be bacterial, and the bacterium most suspect was obviously Clostridia, as there was some similarity to the condition of braxy in sheep. Eventually I interested Mr. N. H. Brooksbank in the condition and he was successful in demonstrating the toxin of *Cl. welchii* Type A. I am not a bacteriologist, but I am given to understand that it is not easy to demonstrate these toxins even in comparatively fresh material, which probably explains why the various laboratories contacted previously through the post had failed to incriminate a specific causal agent.

Since death was usually the first indication of the disease, it was obvious that curative treatment was a very minor consideration and that a vaccine offered the only means of combating the disease. I contacted Evans, Leacher and Webb with a view to having some vaccine prepared, but they informed me that the only time they had used a *Cl. welchii* Type A vaccine the results were disastrous, and they strongly advised against any such attempt.

I have only once successfully treated one of these cases. Two years ago a client rang up at 2.0 a.m. on Christmas Eve. He had just returned from a party and had found a cow down with apparent milk fever. However, when I saw the animal her temperature was 106° F., she was semi-conscious but obviously in great pain, and she groaned continuously. I gave her intravenous calcium with 10 c.c. Dimycin* added. I called again early next morning fully expecting to find the cow dead, but she was conscious and more comfortable although still unable to rise. Further Dimycin was given, and also large doses of Probactol† by mouth. This treatment was continued for several days. Very foul, bloodstained diarrhoea was present for the first few days, but eventually the animal made a complete recovery.

An Unusual Case

I would like to tell you of one very peculiar case which occurred in connexion with this disease. It was on a very small farm inside the Borough of Crewe, and one Sunday morning the cow-man, who had arrived rather late for work, went to switch on the electric motor for the milking machine. As he threw the switch there was a flash and the motor refused to function. He then went round to the shippon containing 10 cows, and to his utter consternation found the first 4 cows in the shippon dead, apparently killed instantly as they lay in the stalls, and the remaining 6 cows in a highly nervous state. To complete the picture, a loose piece of broken wire was dangling down close to one of the tubular stalls. On the face of it, it was an obvious case of electrocution. The Electricity Board was sent for, the police were notified and an intensive investigation was begun. Some 3 hours later, no progress having been made, it occurred to somebody that maybe it would not be a bad idea to call in the veterinary surgeon if only to clear the cattle of suspected anthrax! Since they obviously did not suspect anthrax at all, I think it was probably the knacker's men who suggested it.

When I arrived at the small farm the place was a hive of activity; a couple of harassed looking electricians, one police inspector and 3 or 4 constables, together with 2 local authority representatives from the Sanitary Department, and the knacker-wagon which was waiting to be loaded. Having found the animals free from anthrax, I decided to see the carcases opened at the knacker-yard, and as you will have guessed—although it was a shock to me at the time—all 4 had died of clostridial enteritis. This finding was subsequently confirmed by the Ministry's Veterinary Investigation Laboratory.

I returned to the farm and asked the worried electricians how they were progressing. They said they had been over every inch of the wiring and could not find any possible way in which the cows could have been electrocuted. They were extremely relieved when I assured them that the cattle had not been electrocuted. The police were less easily satisfied, and were obviously sceptical of my story of a killer disease which disposed of 4 cows overnight. They were much more inclined to favour the nice simple theory that some person or persons unknown, with a grudge against the owner, had administered poison to the animals. They duly despatched samples of gut and bowel contents to the Police Laboratory at Preston, and these of course were eventually certified as free from poison.

Summary of the Present Position

That account, apart from being an interesting case and even a somewhat amusing one, does show the unpredictable nature of this disease. Briefly then, a summary of the facts gives this picture:—

(*Concluded overleaf*)

* Dimycin: Streptomycin Sulphate, Dihydro-streptomycin Sulphate, 0.5 gramme of each per 3-c.c. solution. Made by Glaxo.

† Probactol: Suspension containing Phthalyl Sulphacetamide 8.0 per cent. w/v, Sulphadimidine 1.0 per cent. w/v, Kaolin lev. 10.0 per cent., Pectin 1.0 per cent. Made by C. J. Hewlett & Son, Ltd.

The 1958 Annual Congress

A more detailed programme of the 1958 B.V.A. Annual Congress is published overleaf, and below is a list of recommended hotels in Douglas. It is hoped that this programme will attract a good attendance and that the information which follows will be of use to members in making their own arrangements and reservations.

Particulars of methods of travel to the Isle of Man will be published in an early issue, and nearer to the date of the Congress a form of application for Congress tickets, papers, etc., will be circulated with THE VETERINARY RECORD.

HOTEL LIST

Hotel (* Licensed)	Accommodation		Bed and Breakfast		Dinner, Bed and Breakfast; Lunch, Bed and Breakfast		Full Board		Total Capacity	Telephone Number Douglas Ex.
	Single	Double	£ s. d.	£ s. d.	£ s. d.	£ s. d.	£ s. d.	£ s. d.		
*Fort Anne ...	20	27	1 9 6	1 17 6	2 2 0	2 10 0	1 17 6	3 3 0	75	503 and 565
*Peveril	34	20	1 2 6		1 11 0		1 15 0		74	2860
*Villiers	40	70	19 6		1 3 6		1 7 6		180	465
*Belvedere ...	6	27	1 0 0		1 0 0		1 3 6	1 10 0	60	2274
*Sefton	20	20	1 1 0		1 9 6		1 12 6	1 15 0	60	2555
*Central	16	30	1 2 0		1 10 0		1 9 6	1 12 0	76	479
*Empress ...	50	40	1 3 0		1 10 0		1 13 6		130—180	1935
Marlborough ...	4	32	15 0		19 0		1 2 6		68	1954
*Castle Mona ...	10	20	1 5 6		1 13 6		1 19 6		50	481
*Metropole ...	20	80	1 2 6	1 5 0	1 6 6	1 11 6	1 10 0	1 15 0	180	2381
*Sandyford ...	30	32	1 0 0		1 4 0		1 8 0		94	2006
*Waverley (Milnes)	26	60	1 1 0	1 6 0	1 5 6	1 10 0	1 7 6	1 12 6	146	546
*Castletown Golf Links Hotel, Fort Island	26 (18 with bath)	26	1 5 0	1 10 0	1 17 6	2 2 6	1 17 6	2 5 0	78	Castletown Ex. 2201

Transport required—Hotel situated 11 miles from Douglas. Golf extra 4s. 6d. per round. Swimming and Tennis free.

Clostridial Enteritis in Cattle—*Concluded.*

1. The disease is an acute and rapidly fatal gastro-enteritis caused by *Cl. welchii* Type A.

2. It is predominantly associated with the period shortly after calving, although not exclusively confined to this time. It does, however, suggest that parturition acts as a trigger mechanism to start the condition (although this would imply some other "trigger" operating in those cases which occur outside calving time).

3. The incidence of the disease has been increasing steadily over the last 10 to 12 years, and has now reached a really serious position. The knacker-yard assure me that an average of one case a day throughout the year would be a very conservative estimate. To my mind, any disease which can kill some 400 cattle in one small area in a year has reached the stage when it merits the most complete investigation possible.

4. The disease is somewhat seasonal but only, I think, because it is so closely related to the seasonal incidence of calving.

5. It does not appear to be infectious since, with a few exceptions, cases are usually isolated, only one animal on a farm being affected.

6. To the best of my knowledge it is, as yet, confined to Cheshire but I have not sufficient information to be certain on this point.

Although this short account deals with the condition in cattle, I would like to say here that I strongly suspect that it is now spreading to pigs. Since the spring of this year I have encountered a fair number of cases in pigs (usually pigs of 100 to 150 lb. weight) with absolutely no history of illness—simply sudden death which occurs 3 to 4 hours after the animal has taken its last meal. Here again, the *post-mortem* picture is a very acute gastroenteritis which may be so acute that the lumen of the bowel is full of blood and the carcase is bled white. The stomach is always full of food. I have no laboratory confirmation, but the condition does appear to be different from the normal enteritis which is frequently seen in pigs. It has only recently become evident, and the ultra-rapid death and the *post-mortem* appearance of the bowels so closely resembles the condition seen in cattle that I strongly suspect it is the same disease.

B.V.A. Congress, 1958 : Douglas, Isle of Man
September 21st to 26th

PROVISIONAL PROGRAMME

Sunday, September 21st

Time	Congress Social Events, etc.	Scientific Sessions	Tours, Excursions, etc.
	Note.—After the arrival of the Saturday boat from Liverpool, there will be no incoming boat on Sunday.		
p.m. 2.15	(Congress Office open till 6.0 p.m. for registrations —Palace Buildings)		Tour of T.T. Course *or* Angling Excursion
6.30	Choral Evensong, St. Thomas's Church		
8.0	**Council Meeting** (Villa Marina)		
8.30	Lancashire Division **"At Home"** (Villa Marina)		
10.0 to midnight	Film Show in aid of Benevolent Funds (Villa Marina)		

Monday, September 22nd

Time	Congress Social Events, etc.	Scientific Sessions		Tours, Excursions, etc.
a.m. 10.0	**Official Opening of Congress and Civic Welcome** from the Deputy Governor, Isle of Man, and the Mayor of Douglas Followed by :—			
11.0	**Opening of Trade Exhibition**			
12.0	**Annual General Meeting and Presidential Address**			
p.m. 2.30		Metabolic diseases of the ruminant 1. In relation to husbandry *Author :* Mr. L. E. Hughes *Chairman :* *Opener :* 2. Vitamin D in milk Fever *Author :* Mr. C. Weighton *Chairman :* *Opener :* 3. Acetonaemia *Author :* Dr. J. R. Holmes *Chairman :* *Opener :*	Skin conditions met with in small-animal practice *Author :* Mr. J. A. Dall *Chairman :* *Opener :*	Ladies' Excursion to Ramsey via Snaefell. Visit to "Aristoc" stocking factory. Sulby Glen to Tholt-y-Will. Mooragh Park for Tea
5.0		Films (Villa Marina) : "The Veterinary Surgeon in Civil Defence"		
8.0	**Mayor's Reception and Dance** (Villa Marina)			

Tuesday, September 23rd

Time	Congress Social Events, etc.	Scientific Sessions	Tours, Excursions, etc.
a.m. 9.30		Salmonellosis in animals *Author :* Dr. A. Buxton *Chairman :* *Opener :* Nervous diseases in the dog *Author :* Mr. E. M. Pittaway *Chairman :* *Opener :*	
10.0			Ladies' Annual General Meeting, followed by coffee
10.30			
12 noon		**Plenary Session** **Livestock Improvement in Britain** *Author :* Prof. M. M. Cooper *Chairman :* *Opener :*	
p.m. 2.30		**Plenary Session** **Placental Transference of Antibodies** *Author :* Prof. F. W. R. Brambell *Chairman :* *Opener :*	Ladies' Excursion to Peel City and Castle, via St. John's and Tynwald Hill, Fish Hatchery, Michael ; return via Glen Helen. Grainger's Café, Peel, for tea
3.30		The incidence of respiratory diseases of poultry *Author :* Dr. H. P. Chu *Chairman :* *Opener :*	
5.0		Film (Villa Marina) : " Veterinary Anaesthesia "	
8.0 to 1 a.m.	**Ladies' Guild Dance** (Villa Marina)		

Wednesday, September 24th

Time	Congress Social Events, etc.	Scientific Sessions	Tours, Excursions, etc.
a.m. 9.30		A.I. of cattle : Its development and future *Author :* Mr. G. F. Smith *Chairman :* *Opener :* The cystic hyperplasia pyometra complex in the bitch *Author :* Mr. C. Dow *Chairman :* *Opener :*	
10.30			Ladies' Excursion — Port Erin Fish Hatchery, Port St. Mary to Cregneish and the Sound
12 noon		**Plenary Session** **Address by Prof. T. Lloyd Jones** *Author :* *Chairman :* *Opener :*	
p.m. 2.15		Films of general interest **Demonstrations** at I.o.M. Board of Agriculture and Fisheries Experimental Farm 1. Artificial insemination (Mr. G. F. Smith) 2. Meat inspection techniques (Mr. J. M. Olivant) 3. Mycotic dermatitis (Mr. P. K. C. Austwick)	Visit to the I.o.M. Board of Agriculture and Fisheries Experimental Farm for Exhibition of Manx tweeds, sheep, cats, and kipper-making. Afternoon tea provided by the Board at 4.30 p.m.
7.45 for 8.0	**Banquet** (Villa Marina)		

Thursday, September 25th

Time	Congress Social Events, etc.	Scientific Sessions		Tours, Excursions, etc.
a.m. 9.30		Diseases of the external auditory meatus in the dog and cat *Author :* Miss J. O. Joshua *Chairman :* *Opener :*	The role of the veterinarian in public health *Author :* Mr. C. G. Allen *Chairman :* *Opener :*	
10.30				Ladies' Excursion to Manx Museum escorted by R. B. Moore, Esq., O.B.E.
12 noon		**Plenary Session** **The Global Picture of Animal Disease** *Author :* Sir Thomas Dalling *Chairman :*		
p.m. 2.15				Golf Competition (Pulrose Links). Organiser : Mr. R. H. Hawkins
2.30		Disorders of the lactating sow and new-born pig *Author :* Mr. N. H. Brooksbank *Chairman :* *Opener :*	Fore-limb lameness in the horse *Author :* Dr. A. C. Fraser *Chairman :* *Opener :*	Ladies' Excursion via Manx Electric Railway Coastal run to Ramsey (1¼ hours each way) *or* (Weather permitting) Optional Ladies' tour to summit of Snaefell via Mountain Railway
5.0		Film		
8.0	**Reception by Chairman and Members of I.o.M. Board of Agriculture and Fisheries**(GaietyTheatre)			

Friday, September 26th

Time	Congress Social Events, etc.	Scientific Sessions	Tours, Excursions, etc.
a.m. 9.30		The responsibilities of the veterinary surgeon *Author :* Mr. C. C. Stevens *Chairman :* *Opener :*	
10.30			Ladies' Excursion to Castle Rushden
12 noon		Congress Photograph	
p.m. 2.30		Dystokia in Cattle *Author :* Dr. L. G. Anderson *Chairman :* *Opener :*	Ladies' Excursion : Mystery Tour. Afternoon tea provided Ellerslie Farms, Ltd.
5.0	**Adjourned Annual General Meeting and Installation of New President**		
8.0 to 1 a.m.	**President's Reception**		

News and Comment

R.C.V.S. DAY

Wednesday, June 11th, 1958

The response for all the ceremonies has been as great as last year There are still a few vacancies, but the Registrar of the College asks if anyone still desires to attend, that they would let him know by not later than first post, or by telephone, on Monday morning, June 9th.

CAMBRIDGE UNIVERSITY TIBESTI EXPEDITION

An exhibition of photographs taken by members of the Cambridge University Expedition to Tibesti in the Sahara will be on view in the Library of the R.C.V.S. on Wednesday, Thursday, and Friday, June 11th, 12th, and 13th, between 9.30 a.m. and 5 p.m., and admission will be free.

Two of the leaders of this expedition were members of the profession—Mr. A. R. Akester, M.A., VET.M.B., M.R.C.V.S., and Mr. I. A. Silver, M.A., M.R.C.V.S.—and all members of the profession, their families and their friends, are most welcome to attend the exhibition. It is hoped that it will be well supported as a great deal of care and trouble has been taken by members of the Cambridge Veterinary School and the staff of the College in presenting the material.

Ordinary library facilities will therefore be suspended for the whole of the week beginning June 9th and not only on Monday, Tuesday, and Wednesday as announced in a previous issue of THE RECORD.

NEW PHARMACEUTICAL RESEARCH LABORATORIES

The building of new pharmaceutical research and development laboratories, factory, and offices for Smith Kline and French Laboratories Ltd. has begun at Welwyn Garden City. These manufacturers produce nearly all the country's requirements for nitrofurans, which are used extensively in veterinary medicine.

The new establishment, which will replace the present one at Camberwell, London, has been designed to allow for both horizontal and vertical expansion, and exemplifies the constant endeavour of leading pharmaceutical houses to maintain the advance in pharmaceutical research and development.

Leptospirosis in Pigs—*Concluded.*

OKELL, C. C., DALLING, T., & PUGH, L. P. (1925). *Vet. J.* **81**. 3.

SUTHERLAND, A. K., SIMMONS, G. C., & KENNY, G. C. (1949). *Aust. Vet. J.* **25**. 197.

THIEL, P. H. Van. (1948). "The Leptospiroses." Universitaire Pers. Leiden.

VAN DER HOEDEN, J. (1955). *J. comp. Path.* **65**.

———. (1956). *J. inf. Dis.* **98**. 33.

WILLIAMS, H. R., MCCROAN, J. E., STARR, L. E., & WARD, M. K. (1953). *Clin. Res. Proc.* **1**. 97.

YORK, C. J., & BAKER, J. A. (1953). *Amer. J. vet. Res.* **14**. 5-8.

———. (1957) *Vet. med.* **52**. 563.

DISTINGUISHED SERVICE AWARD

The following is an extract from the *London Gazette* of Tuesday, May 20th, 1958:—

"The Queen has been graciously pleased to give orders for the following promotions in, and appointments to, the Most Excellent Order of the British Empire, in recognition of distinguished service in Malaya for the period August 31st to December 31st, 1957:—

To be Additional Officer of the Military Division of the said Most Excellent Order:—

Lt.-Col. John Clabby, M.B.E. (57804), Royal Army Veterinary Corps."

THE REGISTER OF VETERINARY SURGEONS

The name of William Gerald de Saram, B.V.SC. (QUEENSLAND), c/o Royal Bank of Canada, Montreal, Canada, was entered on the Commonwealth List of the Register of Veterinary Surgeons on May 28th, 1958.

PERSONAL

At a congregation held on May 24th the degree of Ph.D. was conferred on **R. V. Short.** The title of Dr. Short's thesis was "Progesterone in Tissues and Body Fluids."

Births

BROWN.—On June 1st, 1958, to Rosa (*née* Smith), wife of James A. Brown, M.R.C.V.S., of Invergordon, Ross-shire, a daughter, Sally Mary.

LEWIS.—On May 23rd, 1958, to Violet, wife of G. B. Lewis, B.SC., M.R.C.V.S., of Dell Road, Campbeltown, Argyll, a daughter.

STEVENSON.—On June 2nd, 1958, to Jean, wife of R. B. Stevenson, B.SC., M.R.C.V.S., of Amery Farm, Alton, Hants, a daughter, Fiona Jean.

Engagements

CATHCART—REDMAN.—The engagement is announced between Robert Austen Cathcart, B.SC., M.R.C.V.S., eldest son of Mr. and Mrs. H. P. Cathcart of Broughty-Ferry, Dundee, and Dorothy Anne Redman, elder daughter of Mr. A. E. Redman of Farnham and Mrs. A. E. Redman of Lower Bourne, Farnham.

FEATHERSTONE—MALE.—The engagement is announced between Peter Featherstone, B.V.SC., M.R.C.V.S., of St. Michael's Mount, Melton, only son of Mr. L. Featherstone of Pontefract, to Christine, twin daughter of Mr. and Mrs. H. Male of Driffield.

Marriage

RATCLIFFE—MILWARD.—On May 24th, 1958, at the Old Parish Church, Llandrindod Wells, Radnorshire, James Ratcliffe, B.V.M.S., M.R.C.V.S., of Farnworth, Lancashire, to Edith Margaret Milward, L.R.A.M., of Llandrindod Wells; future address: Greenway Cottage, Cross Gates, Llandrindod Wells, Radnorshire.

COMING EVENTS

June

11th, 12th and 13th (Wed., Thurs. and Fri). R.C.V.S. Committee and Council Meetings in London.

11th (Wed.). 115th Annual General Meeting of the Royal College of Veterinary Surgeons at 10, Red Lion Square, London, W.C.1, 3.45 p.m.

Annual General Meeting of the Victoria Veterinary Benevolent Fund at 10, Red Lion Square, W.C.1, 10.15 a.m.

Meeting of the North Wales Division at Bryn Adda, Dolgellau, 2 p.m.

12th (Thurs.). Annual Meeting of the R.A.V.C. Golfing Society at the North Hants Golf Club, Fleet, 10 a.m.

13th (Fri.). Annual Dinner of the Royal Army Veterinary Corps Officers' Club at Grosvenor House, Park Lane, London, W.1.

Summer Meeting of the North of Ireland Veterinary Association at the Royal Hotel, Omagh, 7 p.m.

17th (Tues.). Meeting of the Western Counties Veterinary Association at the Hotel Bristol, Newquay, 2.30 p.m.

20th (Fri.). Meeting of the Society for the Study of Animal Breeding at the New Forest Hall, Brockenhurst, Hants, 10.30 a.m.

Annual General Meeting of the Lincolnshire and District Division at the Eastgate Court Hotel, Lincoln, 2.15 p.m.

Annual General Meeting of the Sussex Veterinary Society in the Old Ship Hotel, Brighton, 2.30 p.m.

25th (Wed.). Summer Meeting of the Herts and Beds Veterinary Society.

September

21st to 26th (Official Opening, Monday, September 22nd). 76th Annual Congress of the British Veterinary Association in Douglas, Isle of Man.

BRITISH VETERINARY ASSOCIATION QUARTERLY MEETINGS IN LONDON

Wednesday, July 9th—No. 7, Mansfield Street

10.00 a.m. Veterinary State Medicine Committee.
11.30 a.m. Parliamentary and Public Relations Committee.
2.00 p.m. Finance Sub-committee.
3.45 p.m. Home Appointments Committee.

Thursday, July 10th—No. 7, Mansfield Street

9.30 a.m. Small-Animals Committee.
11.30 a.m. Farm Livestock Committee.
2.15 p.m. General Purposes and Finance Committee.

Friday, July 11th—Connaught Rooms, Gt. Queen Street, W.C.2

10.15 a.m. Council.

R.C.V.S. EXAMINATIONS

Wednesday, June 25th, and Thursday, June 26th. M.R.C.V.S. Written Examinations.

Friday, June 27th. M.R.C.V.S. Practical and Oral. Examinations commence (Panel " B ").

Monday, June 30th. M.R.C.V.S. Practical and Oral Examinations commence (Panel " A ").

ADDRESSES OF DISEASE INFECTED PREMISES

The list given below indicates, first the county in which are situated the premises on which disease has been confirmed, followed by the postal address and date of outbreak.

Anthrax

Beds. Redhills Farm, Toddington, Dunstable (May 28).

Fowl Pest

Cambs. The Bungalow, Station Road, Manea, March; 5, Council Houses, Upware, Ely (May 24); Lister Cottage, Little Thetford (May 26); Bedwell Hey, Little Thetford, Ely (May 28).

Hants. Stoneycroft, Grosvenor Road, Soldridge Medstead, Alton (May 23); Glendown, Winchester Road, Ropley, Alresford; The Chequers, Ropley (May 24); No. 2, The Dene, Ropley, Alresford (May 28).

Sussex. Adur Vale, Small Dole, Henfield (May 26); Elfin Hollow, Vines Cross, Horam (May 29).

Swine Fever

Berks. Priors Court Farm Cottages, Chieveley, Newbury (May 24).

Ches. Somerford Park Farm, Somerford, Congleton (May 24); Elms Farm, Calveley, Tarporley (May 27).

Derbys. The Beeches, Chapel Street, Spondon (May 27).

Dorset. Parsonage Farm, Mappowder, Sturminster Newton (May 29).

Essex Vicarage Farm, Dovercourt, Harwich (May 22); Burghey Brook Farm, Rivenhall, Witham (May 28).

Lancs. Lower House Farm, Lower House Lane, West Derby, Liverpool (May 24).

Leics. Woodside Farm, Barkestone-le-Vale (May 22); Kirkby House Farm, Kirkby Mallory; Bush House Farm, Great Easton, Market Harborough; Hill Farm, Botcheston, Desford (May 28).

Norfolk. 66, Brandon Road, Watton, Thetford; The Bungalow, Tuttington, Aylsham, Norwich (May 24); Bobby Hill Farm, Wattisfield, Diss (May 27).

Oxford. Charity Farm, Stonesfield (May 29).

Shrops. Manor Farm, Preston Gubbalds, Shrewsbury (May 24); Burwood Farm, Siefton Batch, Craven Arms (May 27).

Somerset. Croft Farm, Mark, Highbridge (May 22); Manor Farm, Wellow, Bath (May 24); Chatleys Farm, Limpley Stoke, Bath; Stapleton Mead Farm, Stapleton, Martock (May 27); Bawns, Chilton Polden, Bridgwater (May 29).

Staffs. Lower Lane Farm, Chorley, Lichfield (May 24); Chorley Place, Chorley, Lichfield (May 27).

Suffolk. Sandpits Farm, Badwell Ash, Bury St. Edmunds (May 22); Green Farm, Woolpit, Bury St. Edmunds (May 24); The Hall, Hemingstone, Ipswich (May 28); Lux Farm, Playford, Ipswich (May 29).

Surrey. Rectory Farm, Lightwater (May 22).

Sussex. Piggeries at Rose Cottage Blds., Chiddingley, Lewes (May 28).

Warwicks. The Sycamores, Walmley Road, Walmley, Sutton Coldfield; Marlborough Farm, Camp, Kineton (May 22); Danzey Farm, Tamworth-in-Arden; Burland House Farm, Oxhill (May 24); Croxhall Farm, Nether Whitacre, Whitacre Heath, Birmingham; Oak Farm, Walmley Ash Road, Walmley, Sutton Coldfield (May 28).

XUM

Letter to the Editor

The views expressed in letters addressed to the Editor represent the personal opinions of the writer only and their publication does not imply endorsement by the B.V.A.

SCIENTIFIC

Dow ET-57 and Warble-flies

Sir,—Following favourable reports of the efficacy of Dow ET-57 ("Trolene," O,O, dimethyl 0-2, 4,5 trichlorophenyl phosphorothioate) against warble-flies in the U.S.A. (McGregor & Bushland, 1957; Adkins, 1957; Raun & Herrick, 1957a, b; the technical publication "Trolene" by the Dow Chemical Company, and other reports), supplies of the drug were obtained through the courtesy of the manufacturing company and field trials were arranged at three Veterinary Investigation Centres in England and Wales.

Initial tests at Weybridge had indicated that calves could be given oral doses of between 205 and 337 mg. per kilo without serious toxicity being evident, although transitory symptoms—salivation, scouring, and enhanced rates of respiration were observed, particularly at the highest dose. In the field, trials were confined to young (non-milking) stock, the animals being given the drug at the rate of 100 mg. per kg. as a bolus or in watery suspension. In some instances the rate of dosage was only approximately followed, being calculated to the nearest half of a 15-gramme bolus. In others, the dose was calculated more accurately, the bolus being broken down and the drug with vehicle given as a suspension.

In some instances, which could not be related to overdosing, alarming symptoms of toxicity, similar in general character to those observed with overdosed calves at Weybridge, were seen up to 24 hours after dosing, but the effect was transitory and no further ill-effects have been reported.

Animals were dosed on a single occasion, on different dates between November and January, with excellent results. Thus, of 20 animals dosed in early January in one herd, four showed evidence of infestation whereas of 16 untreated animals 15 developed warbles. There was, moreover, a very marked difference in the degree of infestation. Thus the four treated animals when examined in March had a total of 18 warbles whereas the untreated animals showed a total of 418. Warbles in the treated animals were, at least in some instances, relatively undeveloped and might not have matured.

It is apparent, therefore, that for the first time a systemic insecticide effective against *Hypoderma* is available. Dow ET-57 has in addition been recommended for use against demodectic mange (Sanger, 1958) and against helminths (Worley, 1957). These claims are being investigated at this Laboratory.

At present full acceptance of the drug for field use against warbles is qualified by the appreciation that it belongs to a group of chemical substances (organic phosphorus compounds) which are often markedly toxic. There is good evidence that Dow ET-57 is one of the less toxic members of the group but further work is required, both to assess its toxicity under a variety of conditions and to ascertain its best mode of use under field conditions in Britain.

Meanwhile there is a likelihood that other systemic insecticides for the control of warble-flies may become available and with this in mind recent work in the Parasitology Department at this Laboratory has been concerned with the development of techniques for the implantation of *Hypoderma* larvae in experimental animals. This work, which will be reported later, should lead to the development of a test which will allow drugs to be assessed for their anti-warble-fly activity with much more efficacy than has been possible in the past.

Acknowledgment is due to the Veterinary Staffs of the Investigation Centres of Aberystwyth, Reading, and Starcross where these field trials were organised.

Yours faithfully,

S. BRIAN KENDALL.

Ministry of Agriculture, Fisheries and Food,
Central Veterinary Laboratory,
New Haw,
Weybridge,
Surrey.

May 15th, 1958.

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FOOT-AND-MOUTH DISEASE

Foot-and-mouth disease was confirmed on May 26th among cattle at Upper Rawcliffe with Tarnacre, Nr. Preston, Lancashire. An area extending for approximately 10 miles around the infected premises has been declared to be an Infected Area.

ADVERTISER'S ANNOUNCEMENT

EVANS MEDICAL SUPPLIES LTD. have pleasure in announcing the availability of pure, stabilised, crystalline trypsin under the trade name of TRYPVET NOVO. The adoption of this new title and its presentation as a veterinary speciality now enables this enzyme to be used as an economical routine adjunct to antibiotic therapy in the treatment of mastitis, as well as in the treatment of localised necrotic conditions.